

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS:

Please amend the claims as follows:

1. An isolated ~~DNA~~ nucleic acid molecule comprising a sequence of nucleotides encoding a human calcium sensitive potassium channel subunit protein designated $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit protein, wherein said protein comprises the amino acid sequence as set forth in SEQ ID NO:2.

2. (Canceled)

3. The ~~DNA~~ isolated nucleic acid molecule of claim 1 comprising a nucleotide sequence as set forth in SEQ ID NO:1 ~~selected from the group consisting of: SEQ.ID.NO.:1, 3, 5, 7, 9, and 20.~~

4. The isolated nucleic acid molecule of claim ~~2~~ 4, wherein said nucleotide sequence comprises a coding portion from nucleotide position 271 to nucleotide 978 of SEQ ID NO: ~~1~~ 1 ~~DNA of claim 1 comprising a nucleotide sequence selected from the group consisting of: positions 271-975 of SEQ.ID.NO.:1, positions 341 to 1171 of SEQ.ID.NO.:3, positions 796 to 1566 of SEQ.ID.NO.:5, positions 869 to 1693 of SEQ.ID.NO.:7, and positions 457 to 1293 of SEQ.ID.NO.:9.~~

5. (Canceled)

6. (Currently amended) An expression vector comprising the nucleic acid molecule ~~DNA~~ of claim 1.

7. (Currently amended) A recombinant host cell comprising the ~~DNA~~ nucleic acid molecule of claim 1.

8. (Currently amended) An isolated and substantially pure human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit protein comprising an amino acid sequence as set forth in SEQ ID NO:2.

9. (Canceled)

10. (Canceled)

11. (Canceled)

12. (Currently amended) ~~A~~ An isolated and substantially pure polypeptide having at least 80% sequence identity to the protein of ~~claim 9~~ claim 8 when measured by BLAST or FASTA.

13. (Withdrawn) An antibody that binds specifically to a human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit protein; or that binds specifically to the $\beta 3$ subunit family of proteins by binding to the conserved core.

14. (Canceled)

15. (Withdrawn) A method for identifying substances that bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins comprising:

(a) providing cells expressing a calcium sensitive potassium channel containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

(b) exposing the cells to a substance that is not known to bind calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

(c) determining the amount of binding of the substance to the cells;

(d) comparing the amount of binding in step (c) to the amount of binding of the substance to control cells where the control cells are substantially identical to the cells of step (a) except that the control cells do not express human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

where if the amount of binding in step (c) is greater than the amount of binding of the substance to control cells, then the substance binds to calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins.

16. (Withdrawn) A method of identifying substances that bind calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins and thus are likely to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins comprising:

(a) providing cells expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

(b) exposing the cells to a compound that is known to bind to the calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

(c) determining the amount of binding of the compound to the cells in the presence and in the absence of a substance not known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

where if the amount of binding of the compound in the presence of the substance differs from that in the absence of the substance, then the substance binds calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins and is likely to be an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins.

17. (Withdrawn) A method of identifying activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins comprising:

(a) recombinantly expressing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins or mutant human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins in a host cell so that the recombinantly expressed human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins form calcium

sensitive potassium channels by forming heteromers with other calcium sensitive potassium channel subunit proteins;

(b) measuring the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence and in the absence of a substance suspected of being an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins;

where a change in the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel $\beta 2$, $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunit proteins.

18. (Withdrawn) A method of identifying DNA sequences in the $\beta 3$ gene that promote, enhance, or repress gene transcription comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector;

(c) comparing the abundance of the reporter protein in the cells of step (b) to the abundance of the reporter protein in cells transfected with the vector without fragments of the promoter region of the $\beta 3$ gene;

where fragments of the promoter region of the $\beta 3$ gene which increase the abundance of the reporter protein in the absence of other promoter elements only in cells which endogenously express $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunits are promoter elements; sequences which decrease the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which do not endogenously express $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunits are repressor elements; and sequences which increase the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which endogenously express $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunits are enhancer elements.

19. (Withdrawn) The method of claim 18 where the vector contains promoter or enhancer sequence elements which function independently of the fragments of the promoter region of the $\beta 3$ gene.

20. (Withdrawn) The method of claim 18 where the abundance of the reporter protein is normalized with respect to the fraction of transfected cells.

21. (Withdrawn) A method of identifying DNA sequences in the $\beta 3$ gene that promote, enhance, or repress gene transcription comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells; and

(b) separating the incubation on a gel;

where fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene that migrate differently in a gel ('undergo a shift') after incubation with nuclear extracts from cells are DNA sequences which bind nuclear factors which promote, enhance or repress $\beta 3$ gene expression.

22. (Withdrawn) The method of claim 21 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the method of claim 18.

23. (Withdrawn) The method of claim 21 where the cells express $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunits.

24. (Withdrawn) The method of claim 21 where the cells do not express $\beta 3a$, $\beta 3b$, $\beta 3c$, or $\beta 3d$ subunits.

25. (Withdrawn) A method of identifying nuclear factors involved in $\beta 3$ gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with cloned or purified transcription factors and separating the incubation on a gel;

where factors which bind $\beta 3$ gene promoter sequence elements will induce a shift in the migration of the radiolabeled DNA fragments, and are involved in $\beta 3$ gene transcription regulation.

26. (Withdrawn) The method of claim 25 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the methods of claim 18 or 21.

27. (Withdrawn) A method of identifying transcription factors involved in $\beta 3$ gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

(b) adding an antibody that specifically recognizes a single transcription factor or a family of transcription factors to the incubation of step (a), followed by separating the incubation on a gel;

where a super-shift in mobility of the double stranded DNA in step (b) as compared to step (a) indicates that a transcription factor recognized by the antibody binds the double stranded DNA.

28. (Withdrawn) A method of identifying clones encoding nuclear factors involved in $\beta 3$ gene transcription regulation by cloning comprising:

(a) screening an expression library with radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436)

(b) determining which clones of the library bind the radiolabeled fragments of double stranded DNA;

(c) amplifying and sequencing the clones of step (b).

29. (Withdrawn) The method of claim 28 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the methods of claim 18 or 21.

30. (Withdrawn) A method of identifying nuclear factors involved in $\beta 3$ gene transcription regulation by cloning comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating phage expressing cDNA encoded fusion proteins at their surface with the matrix;

(c) removing phage that do not bind to the matrix by washing;

(d) eluting phage bound to the matrix with excess fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene;

where the phage eluted in step (d) encode nuclear factors involved in $\beta 3$ gene transcription regulation.

31. (Withdrawn) The method of claim 30 where the DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the methods of claim 18 or 21.

32. (Withdrawn) The method of claim 30 where the phage eluted at step (d) are amplified and sequenced.

33. (Withdrawn) A method of identifying nuclear factors involved in $\beta 3$ gene transcription regulation comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating nuclear extracts from cells with the matrix;

(c) washing non-binding proteins from the nuclear extract from the matrix;

(d) eluting bound proteins from the matrix with excess double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene;

where the eluted proteins from step (d) are nuclear factors involved in $\beta 3$ gene transcription regulation.

34. (Withdrawn) The method of claim 33 further comprising separating the eluted proteins from step (d) on a gel and staining the gel to test for purity of the eluted proteins.

35. (Withdrawn) The method of claim 34 further comprising sequencing the proteins that have been separated on the gel.

36. (Withdrawn) The method of claim 34 further comprising immunological analysis of the proteins that have been separated on the gel with antibodies directed towards known transcription factors to identify the eluted proteins by western blot or immunoprecipitation.

37. (Withdrawn) The method of claim 33 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the methods of claim 18 or 21.

38. (Withdrawn) A method of identifying nuclear factors involved in $\beta 3$ gene transcription regulation by cloning comprising:

(a) constructing a yeast strain that contains a few to several copies of a fragment of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) preceding a cDNA encoding a reporter protein;

(b) constructing a cDNA library from cells in a vector that allows formation of fusion proteins encoded by the inserted cDNA and a transcription activation domain;

(c) transforming the library of (b) into the yeast strain of (a) and isolating colonies of yeast displaying expression of the reporter protein.

39. (Withdrawn) The method of claim 38 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the $\beta 3$ gene are identified by the methods of claim 18 or 21.

40. (Withdrawn) The method of claim 38 further comprising purifying the vectors from the isolated colonies and sequencing the cDNA in the vectors.

41. (Withdrawn) A method of identifying substances that enhance or inhibit the rate of transcription of the $\beta 3$ gene comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the $\beta 3$ gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector in the presence and absence of a compound;

where (1) if the presence of the compound decreases the abundance of the reporter protein, then the compound is a substance that inhibits the rate of transcription of the $\beta 3$ gene; (2) if the presence of the compound increases the abundance of the reporter protein, then the compound is a substance that enhances the rate of transcription of the $\beta 3$ gene.

42. (Withdrawn) The method of claim 41 further comprising a control in which the effect of the compound on the abundance of the reporter protein in control cells is measured, where the control cells are cells that are essentially the same as the cells of step (b) except that the control cells have been transfected with a vector that lacks fragments of the promoter region of the $\beta 3$ gene.

43. (New) A recombinant host cell comprising a heterologous human calcium sensitive potassium channel subunit protein, wherein said calcium sensitive potassium channel subunit protein is encoded by a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides as set forth in SEQ ID NO: 1.

44. (New) A recombinant host cell comprising a heterologous human calcium sensitive potassium channel subunit protein, wherein said calcium sensitive potassium channel subunit protein is encoded by a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides that encode the amino acid sequence as set forth in SEQ ID NO: 2.

45. (New) A method of producing the recombinant protein of claim 8, comprising:

- (a) inserting a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO:2 into an expression vector;
- (b) transferring the expression vector into a host cell;
- (c) culturing the host cell under conditions appropriate for amplification of the vector and expression of the protein; and
- (d) harvesting the protein.

Status of Claims:

Claims 1- 42 were pending.

Claims 13 and 15-42 have been withdrawn.

Claims 1, 3, 4, 6, 7, 8 and 12 have been amended herein.

Claims 2, 5, 9, 10, 11 and 14 have been canceled without prejudice.

Claims 43-45 are newly added.

Claims 1, 3, 4, 6-8, 12 and 43-45 are presented for reconsideration.